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Substrate Pretreatment can Reduce the Alcohol Requirement During Biodiesel Production Via in Situ Transesterification

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Abstract The ability of physical pretreatment of the feedstock to reduce the alcohol requirement for high yield fatty acid methyl esters (FAME) production during the in situ transesterification of soybeans was investigated. Four physical treatments were studied: (a) dehulling and flaking, (b) dehulling, flaking and passage through a twin screw extruder, (c) passage through an expander type extruder, or (d) conversion to a flour-like consistency (1–10 μm particles) via disruption in a Pulsewave disintegrator. Following drying, optimal reaction conditions for high yield, room temperature, in situ transesterification of the lipid-linked fatty acids resident in these materials were determined. Expander and Pulsewave pretreatment did not substantially reduce the amount of methanol required for high level FAME production below that required for flaked soybeans. However, the combination of flaking, extrusion and drying achieved a minimum nearly threefold reduction in the methanol requirement compared with that for soybeans that had been only flaked and dried. The flaking/extrusion/drying regime resulted in a minimum optimal molar ratio for (methanol/substrate fatty acid) of 9:1 for effective in situ transesterification. This is a 20-fold reduction in methanol usage compared to the previously reported ratio of 181:1 for the use of flaked-only soybeans. (Haas et al. in J Am Oil Chem Soc 81:83–89, 2004).

Keywords Biodiesel · Fats and oils utilization · Fatty acid ester · In situ transesterification · Transesterification

Introduction

‘In situ’ esterification/transesterification refers to the conversion to simple alkyl esters of free fatty acids and glyceride-linked fatty acid esters resident in a biological material. Reactions conducted in this manner are simple, involving the incubation of an alcohol, an inorganic catalyst and a lipid-bearing material at ambient pressure and moderate temperatures. In contrast to conventional methods of production of simple monoalkyl fatty acid esters, the need for the extraction and refining of a lipid before transesterification is eliminated since acylglycerols and fatty acids (FA) are reacted directly within the biological material. The product esters diffuse into the liquid phase of the reaction, from which they can be readily recovered.

The use of this approach has been described for the synthesis of fatty acid methyl esters (FAME), for potential use as biodiesel, from the lipids in sunflower seeds [1–3], rice bran [4–6] and soybeans [7]. In these reports sulfuric acid was employed as catalyst in reactions generally conducted at the reflux temperature of the systems, approximately 65 °C. Using alkaline catalysis, we have further characterized the system, identifying optimum conditions for near-quantitative transesterification of several different substrates [8, 9], demonstrating that substrate moisture levels influence the amount of alcohol required for high degrees of reaction [10], establishing that the FAME product meets the official specifications for biodiesel adopted by the American Society for Testing and Materials [10], and that the lipid-depleted high protein meal coproduct of the transesterification of soy flakes is an acceptable dietary component for trout [11].

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However, the general utility of the in situ transesterification approach as described to date is hindered by a high alcohol requirement. The transesterification of a lipid preparation typically proceeds rapidly and to high degrees of conversion at an alcohol/FA molar ratio of 2:1 [12]. In contrast, for the in situ alkaline transesterification of soybeans this ratio was 181:1 [8]. Drying the feedstock was shown to reduce this ratio to 75:1 [10]. All but one fatty acid molar equivalent of the alcohol remains unreacted after the reaction and can be recovered for further use. However, the energy requirements for the required distillation and condensation render the entire transesterification process economically noncompetitive [13].

It has been reported that mechanical treatment of the substrate can increase the recovery of oil during the enzyme-assisted aqueous extraction of oil from soybeans [14]. We report here the results of an examination of the possibility that pretreatment can also reduce the alcohol requirement during in situ transesterification.

Experimental Procedures

Chemicals, Routine Methods

The moisture content of soybeans was determined by drying to constant weight in a convection oven (77 °C). Acylglycerol contents were determined by extraction with hexane for 4.5 h in a Soxhlet apparatus followed by removal of the solvent under vacuum and determination of the triacylglycerol content of the hexane extract by HPLC (below). Moisture and lipid contents reported are the averages of two independent determinations. Replicate values differed by no more than 5%. To calculate maximum theoretical FAME mass yields, (a) the average molecular weight (MW) of a soybean oil triacylglycerol was calculated, based on the published average MW of a soybean FA [15]; (b) this MW, in combination with data on the total oil content of the soybean substrate as determined by exhaustive hexane extraction, allowed calculation of the number of moles of triacylglycerol in the substrate; (c) the theoretical maximum molar yield of FAME was taken as three times the molar content of triacylglycerols; (d) this maximum molar FAME yield was converted to a maximum theoretical mass FAME yield using the average MW of a soybean fatty acid [15] to calculate the average MW of a soybean FAME.

Soybean substrates were dried as described above for whole soybeans till desired moisture levels, determined gravimetrically, were attained

A mixture of FAME whose composition reflected the FA content of soy oil (RM-1) was the product of Matreya, Inc. (Pleasant Gap, PA, USA). Organic solvents were B&J Brand™ high purity grade (Burdick & Jackson, Inc., Muskegon, MI, USA). Sodium methylate (30 wt% in methanol) was the generous gift of Evonik Degussa GMBH (Niederkassel, Germany).

Feedstock Pretreatments

Soybeans were pretreated as indicated in advance of in situ transesterification:

Flaked. Soybeans that had been dehulled and passed between flaking rollers, yielding flakes with a thickness of approximately 0.4 mm, as for industrial hexane extraction, were generously provided by Archer Daniels Midland Co. (Decatur, IL, USA). Moisture and lipid contents were 2.8 and 23.0 wt% respectively. The moisture level was reduced to less than 0.1% by drying in a convection oven prior to transesterification.

Flaked and Extruded (‘*flaked/extruded*’). Soybeans were cracked, dehulled, conditioned, flaked and extruded as previously described [14]. Extrusion was conducted using a twin-screw extruder (18 mm screw diameter, Micro 18; American Leistritz Extruders, Somerville, NJ, USA) operating with a 100 °C barrel temperature and a high shear screw rotational speed of 100 rpm. The extruded material was in the shape of cylinders with a 4-mm diameter and approximately 6 mm long, and these had a moisture content of 10.3 wt% and a triacylglycerol content of 22.4 wt% upon receipt. The moisture level was reduced to less than 0.1% by drying in a convection oven prior to in situ transesterification.

Expanded and milled (‘expanded/milled’). Raw, dehulled soybeans were ground in a hammer mill to pass a 4.8 mm screen and fed through an Insta-Pro Series 2000 expander/extruder (Insta-Pro, Des Moines, IA, USA) operating at 700 psi and 150–157 °C. Process time in the expander was approximately 30 s. The resulting material was tumbled in an air stream to reduce the temperature to ambient and passed through a family grain mill with grain mill attachment (Pleasant Hill Grain, Hampton, NE, USA) at its finest setting. The product was a fine meal. 14.4% of its mass was retained by a No. 35 sieve (0.500 mm pore size) and 96.8% was retained by a No. 60 screen (0.25 mm). Moisture and lipid contents were 7.8 and 21.1 wt%, respectively. The moisture level was reduced to 4.6 wt% by drying in a convection oven prior to transesterification.

Pulsewave treated (‘*Pulsewave*’). Dehulled soybeans were disintegrated by processing in a custom disruptor that subjected them to shock waves created by flowing through a housing having alternating rotors and orifice plates [16]. Details of the treatment were not disclosed (W. Hahn, Pulsewave LLC, Centennial, CO, USA). Upon receipt the material contained 2.5 wt% moisture and 22.4 wt% oil. Microscopic examination indicated that approximately

Table 1 Ranges of variables investigated in a central composite experimental design mode to determine the effect of soybean pretreatment on reagent requirements for effective in situ transesterification

Substrate preparation	Variable	Amount/duration				
Flake						
	Sodium methylate (mmole/reaction)	0.50	0.72	1.15	1.58	1.8
	Methanol (mL)	2.0	3.3	6.0	8.7	10.0
	Reaction time (h)	2.5	3.3	5.0	6.7	7.5
Flake, extrude						
	Sodium methylate (mmole/reaction)	0.30	0.50	0.90	1.30	1.50
	Methanol (mL)	1.5	3.0	6.0	9.0	10.5
	Reaction time (h)	1.0	2.0	4.0	6.0	7.0
Expand, mill						
	Sodium methylate (mmole/reaction)	0.50	0.58	0.75	0.91	1.00
	Methanol (mL)	2.0	2.5	3.5	4.5	5.0
	Reaction time (h)	0.50	1.0	2.0	3.0	3.5
Pulsewave						
	Sodium methylate (mmole/reaction)	1.2	3.7	5.7	8.7	10.2
	Methanol (mL)	6.6	7.5	9.3	11.1	12.0
	Reaction time (min)	30	40	60	80	90

80% of the particles were between 1 and 10 μm in size. This material was used without further drying.

Feedstock samples were stored at −20 °C in sealed containers.

Conduct and Optimization of in Situ Transesterification

Pretreated soybean meal (5.00 g) was added to solutions containing the desired amounts of methanol and sodium methylate in screw-capped bottles of capacity at least 5 times the reaction volume. These were mixed by rotary shaking at a speed sufficient to keep the solids well suspended. Reactions were conducted at 23 °C. Following the reaction, bottles were allowed to sit for 15 min at room temperature to allow the solids to settle. The liquid phase was removed and the solids were processed by centrifugation and resuspension in 10-mL aliquots of methanol. Two such washes were sufficient to obtain greater than 90% of the recoverable FAME in reactions using either flaked, extruded/milled, or Pulsewave-treated material; three were required in the case of the flaked/extruded soybeans. The washes were pooled with the reaction liquid, and the FAME content was determined by HPLC.

Central composite response surface design methods [17] were employed to coordinately investigate the effects and interactions of the amount of methanol, the sodium methoxide content, and the reaction time on the yield of FAME. Preliminary studies (data not shown) were conducted to focus the statistically designed work in the region of variable space giving greatest FAME production. Table 1 presents the levels examined for each of the variables. Reaction compositions giving high FAME yields in less than 5 h of reaction were studied, since longer reaction times may not be practical in an industrial setting. For each substrate, 20 reactions were conducted using various combinations of these levels.

Best-fit equations correlating the amounts of FAME produced with the compositions of the reactants were constructed using SAS/STAT software [18]. Numerical analysis of these equations and examination of the corresponding three-dimensional surfaces allowed identification of the conditions predicted to give the maximum FAME yield.

Analytical Methods

Fatty acid methyl esters contents were determined by HPLC on a diol-modified silica column (LiChrosorb 5 Diol, Varian, Walnut Creek, CA, USA) developed isocratically with a mixture of hexane and acetic acid (99.9:0.1 vol%) at a flow rate of 0.5 mL/min. Peaks were detected by evaporative light scattering and quantitated by reference to standard curves constructed with a known pure FAME mixture whose FA composition corresponded to that of soybean oil (RM-1, Matreya, Pleasant Gap, PA, USA). The feedstocks examined here lacked detectable amounts of free fatty acid, and di- and monoacylglycerols.

Results and Discussion

The basis of this work is the postulate that intact cellular structures inhibit the access of transesterification reagents to cellular acylglycerols, and that this is responsible for the large alcohol requirement for high efficiency in situ transesterification. Soybeans were therefore subjected to a number of treatments that were likely to disrupt cellular integrity, possibly facilitating penetration of transesterification reagents and effecting a reduction of the alcohol requirement for successful transesterification.

Statistical experimental design was employed to explore the effect of substrate pretreatment on the amounts of catalyst and methanol, as well as reaction time, required for high efficiency transesterification, and to identify any interactions between variables. The goal, for each type of substrate pretreatment, was to identify the minimum reagent requirements and reaction time predicted to give complete or near complete transesterification. Given their lipid contents, it was calculated that the soybean preparations examined here had maximum potential FAME yields between 1,087 and 1,185 mg. We judge that an efficiency of at least 90% in the conversion of acylglycerol FA to FAME is necessary in order for this approach to be industrially relevant. Therefore, transesterification conditions were sought that were predicted to yield at least 1,000 mg of FAME per reaction. Since previous work has shown that elevated substrate water content increases the methanol requirement of this reaction [10], substrate moisture levels were reduced below 5% for these studies.

In experiments such as these where both reaction volume and catalyst concentration are coordinately varied, we have found the extent of transesterification to be more uniformly related to the amount of catalyst present than to its concentration (Haas and Wagner, unpublished observations). This may indicate that some catalyst is consumed in neutralizing cellular components before an alkalinity sufficient for transesterification is achieved. Therefore, sodium methoxide levels are expressed here as the total amount in the reaction rather than the concentration.

Analysis of FAME production as a function of reaction composition (Table 1) yielded predictive equations correlating these parameters:

Flaked soybeans:

$$\begin{aligned}\text{FAME} = {} & -2334.90 + 175.24V + 3187.38N + 208.25T \\ & - 84.27VN + 2.41VT - 23.47NT - 3.76V^2 \\ & - 888.43N^2 - 14.93T^2 \quad R^2 = 0.97 \end{aligned} \tag{1}$$

Flaked/extruded soybeans:

$$\begin{aligned}\text{FAME} = {} & 926.41 - 5.55V + 6.81N + 56.28T + 4.88VN \\ & - 0.47VT + 8.44NT + 0.74V^2 - 51.38N^2 \\ & - 6.82T^2 \quad R^2 = 0.72 \end{aligned} \tag{2}$$

Expanded/milled soybeans:

$$\begin{aligned}\text{FAME} = {} & -624.56 + 57.59V + 2797.90N + 184.45T \\ & - 298.91VN + 42.88VT - 9.38NT + 19.86V^2 \\ & - 943.77N^2 - 74.59T^2 \quad R^2 = 0.70 \end{aligned} \tag{3}$$

Pulsewave treated soybeans:

$$\begin{aligned}\text{FAME} = {} & 731.24 - 7.30V + 180.27N - 6.27T \\ & + 2.50VN + 0.12VT + 0.25NT - 0.02V^2 \\ & - 20.11N^2 + 0.03T^2 \quad R^2 = 0.96 \end{aligned} \tag{4}$$

where FAME = predicted FAME yield (mg), V = methanol volume (mL), N = amount of catalyst (mmoles), T = reaction time (h, except for Pulsewave data:min).

The reasonably high R^2 values for these equations indicate acceptable degrees of agreement between the data set and the derived predictive equation. The lower R^2 values for Eqs. 2 and 3 correspond to slightly more variation in these data sets between the results of the 6 replicate determinations that were included in each of the central composite experimental designs. However, in all four cases the differences between the lowest and the highest values in these replicates are between 2 and 5% of the mean of the replicates, and the models are thus judged to be accurate representations of reality. In the low R^2 cases, only one or two of the values for the six replicates differ by as much as 5% from the mean, and the variations are thus considered to most probably be due to unavoidable small differences in technique during work up of the samples for analysis.

These equations were used to generate three-dimensional graphs of predicted FAME yield as a function of the amounts of methanol and catalyst at various reaction times. In calculating the methanol content, both neat added methanol and that entering the reactions with the sodium methylate catalyst were accounted for. For the feedstock pretreatments examined here, Figs. 1, 2, 3, 4 display predicted FAME yield surfaces as a function of reaction composition, selected at the earliest reaction time giving high yields of FAME. Table 2 presents reaction conditions for each feedstock pretreatment, identified by numerical analysis of Eqs. 1–4 and Figs. 1, 2, 3, 4, giving FAME yields in excess of 90% of maximum theoretical.

In our initial work in this area, using flaked soybeans with 7.4 wt% moisture, the molar ratio of methanol to acylglycerol FA for high efficiency transesterification was 181:1 [8]. Subsequently the methanol/FA requirement was found to be reduced by drying of the substrate [10]. Given an average MW of 278.2 for soybean oil FA [15] and the 4.0 mL optimum methanol level for the in situ transesterification of dry soy flakes reported here (Table 2) the comparable value is

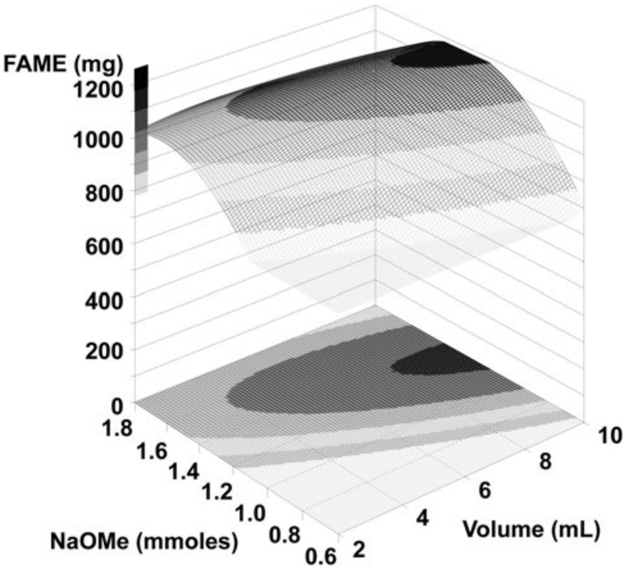

Fig. 1 Predicted FAME yields in the in situ transesterification of flaked soybeans (5 g) as a function of the amounts of sodium methoxide and methanol. Reaction time: 4.5 h. Maximum theoretical FAME yield: 1,180 mg

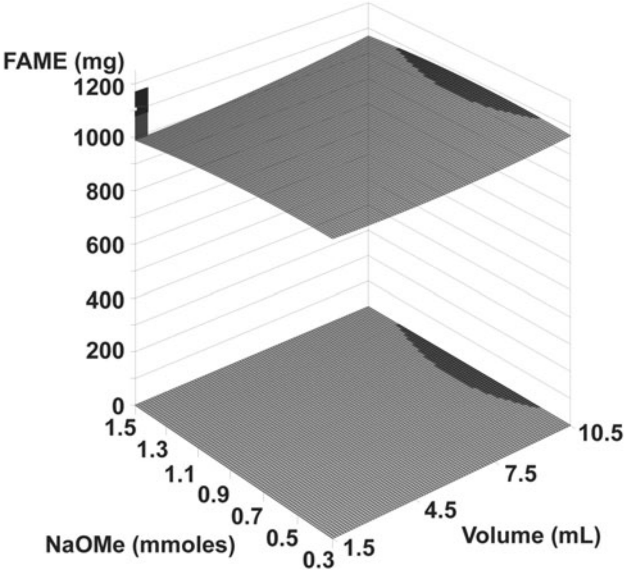

Fig. 2 Predicted FAME yields in the in situ transesterification of flaked/extruded soybeans (5 g) as a function of the amounts of sodium methoxide and methanol. Reaction time: 4.0 h. Maximum theoretical FAME yield: 1,183 mg

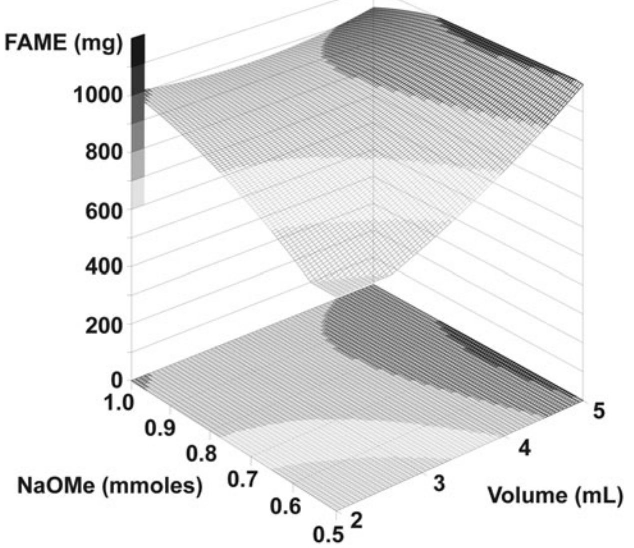

Fig. 3 Predicted FAME yields in the in situ transesterification of expanded/milled soybeans (5 g) as a function of the amounts of sodium methoxide and methanol. Reaction time: 2.5 h. Maximum theoretical FAME yield: 1,087 mg

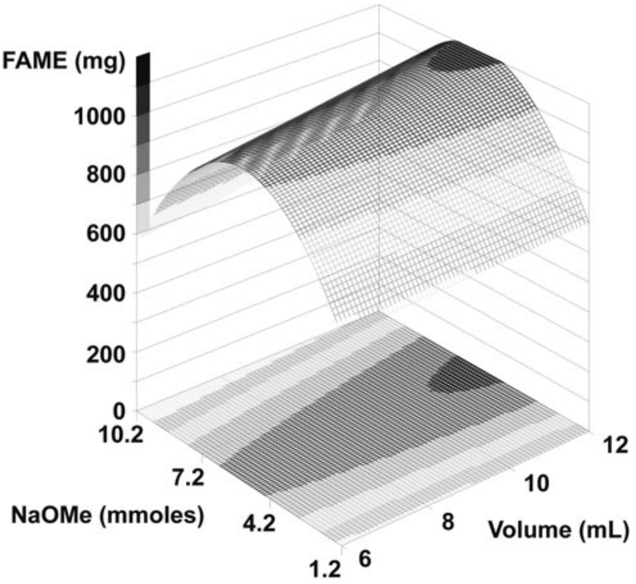

Fig. 4 Predicted FAME yields in the in situ transesterification of soybeans (5 g) treated with Pulsewave disintegration technology, as a function of the amounts of sodium methoxide and methanol. Reaction time: 1.5 h. Maximum theoretical FAME yield: 1,113 mg

calculated to be 25/1, a significant reduction compared to undried substrate.

Two other pretreatments of the soybean feedstock examined, extrusion/milling and pulverization using Pulsewave disintegration technology, gave similar reductions in the methanol requirement for successful transesterification (Table 2). Of possible utility, reactions of material treated by these two methods proceeded more rapidly than those conducted with flaked soybeans, with Pulsewave treated samples requiring only slightly greater than 1/3 the reaction time to achieve maximum FAME levels (Table 2). It is worth noting, however, that the fine powder-like consistency of the Pulsewave treated material could complicate its handling in an industrial setting.

Passage of flaked soybeans through a twin screw extruder gave the greatest reduction in methanol

Table 2 Least volume optimal conditions for >90% maximum theoretical FAME yield in the room temperature in situ transesterification of soybeans (5 gm) subjected to various pretreatments

Pretreatment	Methanol (mL)	Sodium methylate (mmole)	Reaction time to max. yield (h)
Flake	4.0	1.5	4.5
Flake, extrude	<1.5	<0.3	4.0
Expand, mill	4.0	0.8	2.5
Pulsewave	4.0–6.0	4.2	1.5

requirement for successful in situ transesterification. Near theoretical maximum FAME yields were predicted in reactions containing 1.5 mL or less of methanol/5 g of feedstock. The 1.5 mL addition rate corresponds to a methanol/FA molar ratio of approximately 9:1. Thus, compared with the 181:1 methanol/FA ratio reported for flaked soybeans [8], the addition of extrusion and drying procedures effected a very substantial 20-fold reduction in methanol requirement. The 9:1 molar ratio of alcohol over fatty acids approaches the 2:1 ratio that has been shown most effective for the transesterification of refined soybean oil [12]. It is also notable that this pretreatment allowed a fivefold or better reduction in the amount of catalyst required for effective transesterification (Table 2).

Especially for soybeans pretreated by the Pulsewave disintegration process (Fig. 4) but also to a small degree for soybean treated by conventional flaking methods (Fig. 1), increasing amounts of sodium methoxide are predicted by Eqs. 4 and 1, respectively, to reduce the degree of in situ transesterification. We have not investigated the causes of these yield reductions. It is possible that with these substrates, an elevated amount of sodium methoxide modifies the matrix in a manner that reduces contact between substrate and catalyst, or interferes with diffusion of the FAME product into the liquid phase.

We previously constructed a techno-economic model to estimate the per-gallon cost of biodiesel production from refined soybean oil by conventional alkaline transesterification technology [19]. Such models incorporate equipment, reagent, utilities, and associated costs, at scales relevant to industrial operations, to assess the capital and operational costs of a technology. In a similar, although preliminary, analysis of the costs of in situ transesterification we found this methodology, using dried soybean flakes, to have an estimated process cost 24% higher than that for the transesterification of refined soybean oil via conventional technology [13]. The bulk of the higher cost was due to the energy requirements of distillation and condensation steps required to recover the large amount of unreacted methanol. It is possible that the reduction in methanol requirement that has been shown here to result from an extrusion pretreatment of the feedstock could render the in situ transesterification method less costly than conventional methods for biodiesel production, thereby reducing the cost of this renewable fuel. It remains to be determined whether feedstock pretreatment similarly reduces reagent requirements for the in situ transesterification of substrates other than soybeans.

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